Journal of Pharmacological Sciences 127 (2015) 382–390

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Disruption of running activity rhythm following restricted feeding in female mice: Preventive effects of antidepressants

Kazumi Miyawaki [a, *], Hiroaki Araki [a], Hiroyuki Yoshimura [b]

[a] Department of Clinical Pharmacy, Ehime University Graduate School of Medicine, Shitsukawa, Toon-city, Ehime 791-0295, Japan
[b] Behavioral Pharmacology Laboratory, Research Institute for Alternative Medicine, Hinokuchi, Toon-city, Ehime 791-0202, Japan

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form
12 February 2015
Accepted 22 February 2015
Available online 4 March 2015

Keywords:
Wheel-running activity
Circadian rhythm
Chronometrical analysis
Antidepressant drugs
Female mice

ABSTRACT

Biological rhythms are critical in the etiology of mood disorders; therefore, effective mood disorder treatments should address rhythm disturbances. Among the variables synchronized with the light–dark cycle, spontaneous activity in rodents is useful for investigating circadian rhythms. However, previous studies have focused only on the increase of wheel-running activity under restricted feeding conditions, while little information is available on circadian rhythm of running activity. In this study, chronometrical analysis was used to assess whether circadian rhythms during wheel-running are altered by restricted feeding and affected by antidepressant drugs. Wheel revolutions were automatically recorded and analyzed using cosinor-rhythmometry in 8-week old ICR albino mice. When feeding was restricted to 1 h per day (21:00–22:00), wheel-running rhythms were reliably disrupted. Female mice exhibited marked alterations in the pattern and extent of wheel-running beginning on day 1. Subchronic treatment with imipramine or paroxetine, as well as tandospirone and (−)-DOI, prevented wheel-running rhythm disruption. Thus, altering the circadian activity rhythms of female mice on a 1-h feeding schedule may be useful for investigating disturbances in biological rhythms.

1. Introduction

Since the concept of the "animal model" was introduced into psychiatric medicine, various experimental models of mental illnesses, including anxiety, depression and dementia, have been developed and used to screen novel psychotropic drugs, and to understand the brain mechanisms underlying behavioral disorders. However, the disruption of rhythmicity in psychiatric patients remains under-researched: the difficulty of devising a valid animal model, and the complicated statistical analysis required to assess circadian rhythms, may have restricted the use of a chronobiological approach in experimental psychopharmacology. However, depressive disorder is accompanied by alterations in the periodic rhythms of daily life. Indeed, the rhythm of the sleep–wake cycle, which is synchronized with the light–dark cycle, is important in mood disorders and desynchronization with the light–dark cycle is a risk factor for depression (1–3). Females suffering from mood disorders are characterized by a high prevalence of sleep disturbances (4) and irregular eating patterns (5). Although pharmaceutical research has focused on time-dependent drug effects and pharmacodynamics (6–8), information concerning the effects of drugs on desynchronized behavioral patterns remains limited.

Among the variables synchronized with the light–dark cycle, spontaneous activity in rodents is particularly instructive for the investigation of circadian rhythms. When mice and rats are individually housed in cages with running-wheels under restricted feeding conditions (1 h per day), they exhibit excessive wheel-running and decreased food consumption, with activity decreasing markedly after reaching peak wheel revolutions, followed by death (9). In early investigations, Routtenberg (10, 11) termed this phenomenon, characterized by novelty and food-deprivation stressors, as "self-starvation". Páre and Houser (12) emphasized that augmenting running activity itself generated stress, termed by these authors "activity-stress." In addition to the development of gastric mucosal lesions (13, 14), the following physical disabilities, possibly resulting from emaciation, have also been reported and have included atrophy of the thymus and spleen (15), as well as and adrenal hypertrophy (16). The number of wheel revolutions during the light-phase was greater compared with the dark-phase in nocturnal rats maintained on a 1-h feeding schedule (17). Although obtaining accurate information on wheel-running activity rhythms following

* Corresponding author.
E-mail address: miyawaki.kazumi.my@ehime-u.ac.jp (K. Miyawaki).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.02.015

restricted feeding is important, chronometric analysis of variations in running activity, plotted against time, has not been reported.

Circadian rhythm disturbances may be the major determinant of mood disorders (3, 18, 19). The prevalence of mood disorders, including sleeping and eating disturbances, is higher in females than in males; the effectiveness of psychotropic drugs also differs between genders (20, 21). Nevertheless, investigators rarely use female animals due to concerns regarding complicated interactions between endocrine changes during the estrous cycle and drug effects. However, it is important to establish that drugs prescribed to females, including antidepressants, are efficacious and safe.

In the present study, a chronobiological approach was taken to assess whether restricting feeding to 1 h per day in female ICR mice desynchronizes wheel-running activity with respect to the light–dark cycle. Because rhythmometrical, spontaneous wheel-running data is limited, we first analyzed three activity rhythm components (midline estimating statistic of rhythm [MESOR], amplitude, and acrophase) in female and male mice under restricted feeding conditions. We then investigated whether antidepressant drug treatment prevents desynchronization in female mice; current evidence suggests that disturbances in circadian rhythms could be normalized by daily treatment with antidepressants (3, 22). Serotonergic nervous activity in the brain appears to contribute to circadian rhythm regulation in mood disorder patients (3), and to depressive-like states in animals (23, 24). We also investigated whether the desynchronization of running activity rhythms, in response to a 1-h feeding schedule, is altered by subchronic treatment with tandospirone, a 5-HT_{1A} receptor agonist, or (−)-2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT_{2A} receptor agonist.

2. Materials and methods

2.1. Subjects

ICR albino male and female mice (CLEA, Osaka), 6 weeks of age, were habituated to laboratory conditions for at least 2 weeks in a conventional polycarbonate cage (21 × 31 × 14 cm, 10 animals per cage) before the experiments commenced. Mice had free access to food and water. Cage floors were covered with wood shavings, and were cleaned every 4 days. The temperature of the vivarium was maintained at 23 ± 2 °C; a 12/12-h light/dark cycle was automatically controlled (lights on/off at 07:00 and 19:00, respectively). The experiments were conducted in accordance with National Institutes of Health guidelines and the Guide for Animal Experimentation of the Ehime University School of Medicine.

2.2. Apparatus

The apparatus consisted of a running wheel (20.5 cm in diameter) and adjoining stainless-steel cage (7 × 7 × 8 cm) with a wire-mesh bottom (Muromachi Kikai Co. Ltd., Tokyo). A sliding door separated the wheel from the adjoining cage, and mice could take water freely. Each cage was placed on a small plate with wood shavings, but mice did not touch the wood shavings. Wheel revolutions were automatically recorded, using an interface (MSR128-V5, M-System Co. Ltd., Osaka) connected to a computer system, at 1 min intervals. For analysis purposes, intervals were summed to 60 min.

2.3. General procedure

The estrous stage of 8-week-old female mice was determined twice per day (between 09:00–10:00 and 16:00–17:00) using vaginal smears before the experiment. Vaginal smears were collected using a modified Pasteur pipette filled with 0.9% saline solution. We identified each estrous stage microscopically according to the methods described in Bekku et al. (24). When the mice were in the diestrus stage, they were removed from their home cage and placed into a wheel cage, at 17:00 to measure running activity. All mice were habituated to the apparatus for 4 days without food restriction prior to the onset of the experimental feeding conditions. We measured daily body weight at 17:00.

2.4. Experimental design

With the exception of the first experiment, three separate series of experiments were conducted using female mice. The first experiment examined gender differences; 19 males and 15 females were randomly subdivided into *ad libitum* and 1-h feeding groups. The second experiment assessed whether subchronic treatment with imipramine (at 5 or 10 mg/kg) or paroxetine (at 2.5 or 5 mg/kg) attenuated the circadian rhythm desynchronization induced by 1-h feeding conditions. Forty mice were randomly assigned to five groups. The third experiment investigated whether 5-HT_{1A} or 5-HT_{2A} receptors are involved in attenuating the circadian rhythm desynchronization induced by 1-h feeding, using subchronic treatment with tandospirone (at 2.5 mg/kg or 5 mg/kg) or (−)-DOI (at 0.5 mg/kg or 1 mg/kg). Forty-seven mice were randomly assigned to six groups.

2.5. Drug administration

Each drug or vehicle was administered twice per day (at 09:00 and 17:00). Drug treatments were started the day after introducing each mouse to the apparatus, and continued until the experiment was terminated. Imipramine hydrochloride (WAKO Pure Chemical Industries, Ltd., Tokyo) and paroxetine maleate (Sigma, St. Louis, MO, USA) were administered orally. Tandospirone dihydrogen citrate (Sumitomo Pharmaceuticals Co., Ltd., Osaka) was administered orally, while (−)-DOI (Sigma, St. Louis, MO, USA) was administered subcutaneously. All drugs were dissolved in distilled water, except for (−)-DOI which was dissolved in isotonic saline.

2.6. Statistical analysis

The number of wheel revolutions was collected by an interface connected to a computer system; we analyzed these data using cosinor-rhythmometry. Periodic regression analysis (PRA) obtained the best-fit regression curve to the wheel revolutions of every 60 min period for 24 h. A fundamental rhythm with 24-h harmonics was fitted using Fourier analysis, and was assessed by the coefficient of determination (R^2) using least-squares analysis. We calculated the following three parameters (25): MESOR (24-h mean wheel revolutions about which oscillation occurs); amplitude (the distance between MESOR and the highest value of the curve); and acrophase (timing of the highest point, in degrees). Periodic analysis of covariance (PANCOVA) was used to assess group differences in periodic regression curves, according to wheel-running extent (MESOR) and pattern (amplitude and acrophase). A value of $p < 0.01$ was taken to indicate statistical significance. Differences in either extent or pattern were considered statistically significant according to the method described by Hayashi (26). MESOR, amplitude, and acrophase consistency, between the control and experimental groups, was analyzed using the intraclass correlation coefficient (ICC). All data are expressed as means ± *SE*. Multiple comparisons were performed using Bonferroni's multiple comparison tests following ANOVA or ANCOVA.

3. Results

3.1. Comparison between male and female mice

The 1-h mean wheel-running activity revolutions, collected automatically at 1 min intervals throughout 6-day periods, are plotted in Fig. 1. Running activity rhythms in the *ad libitum* feeding group were well-synchronized with the light–dark period in both male (Fig. 1a) and female (Fig. 1c) mice. However, when feeding was restricted to 1 h per day, following the 4-day habituation period, circadian rhythms were disrupted (Fig. 1b and d); female mice ran during either dark or light phases and died suddenly within 3 days of the start of the 1-h feeding schedule. Table 1 shows the parameters for periodic regression curves between the *ad libitum* and 1-h feeding groups in male and female mice. In male mice PANCOVA revealed no significant differences in wheel-running extent or pattern, between the *ad libitum* and 1-h feeding groups during the adaptation period and on day 1 of restricted feeding. However, on day 2, there was a significant group difference ($F_{(1, 42)} = 19.145$, $p < 0.01$). MESOR in the 1-h feeding group was also significantly higher than that of the *ad libitum* group ($p < 0.01$). On day 3, PANCOVA revealed a significant difference in wheel-running extent ($F_{(1, 42)} = 13.969$, $p < 0.01$), and the 1-h feeding group showed a higher MESOR value compared with the *ad libitum* group. In female *ad libitum* mice, there were significant differences in the MESOR between the *ad libitum* and 1-h feeding groups. PRA also revealed higher R^2 values, fitted with 24-h power density, compared with the other periodic components. On day 1, PANCOVA showed that wheel-running extent ($F_{(1, 42)} = 45.169$, $p < 0.01$) and pattern ($F_{(2, 42)} = 11.145$, $p < 0.01$) differed between the *ad libitum* and 1-h feeding group females. MESOR of the 1-h feeding group was significantly higher than that of *ad libitum* group ($p < 0.01$). Moreover, acrophase after 1-h feeding was shifted from 00:03 to 09:09 compared with the *ad libitum* feeding group. Although PANCOVA revealed a significant difference in the wheel-running pattern of female mice, it was unclear which amplitude and acrophase were affected by restricted feeding. To determine this, we evaluated the consistency between the *ad libitum* and 1-h feeding groups using matching coefficients with ICC. Matching coefficients under 1-h feeding differed from those of the adaptation

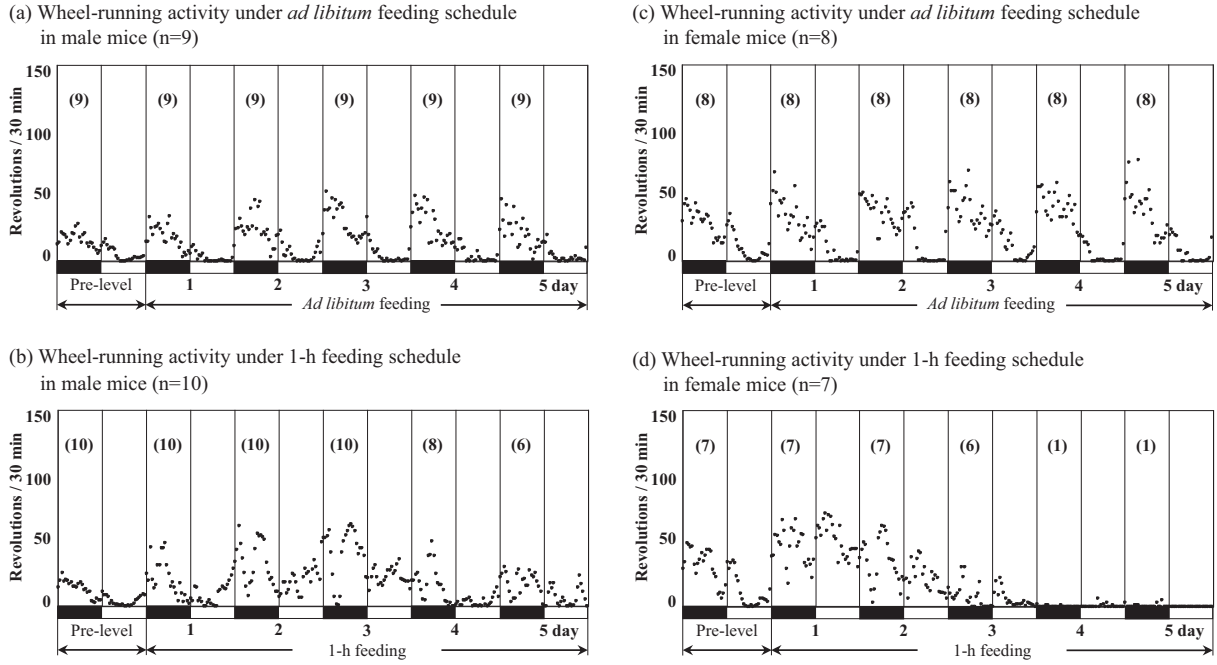

Fig. 1. Comparison of wheel-running activity between male mice (a: *ad libitum* feeding group; b: 1-h feeding group) and female mice (c: *ad libitum* feeding group; d: 1-h feeding group). Each dot shows the mean number of wheel revolutions per 30 min. Black rectangular columns show the dark phase (19:00–07:00 h) and white rectangular columns show the light phase (07:00–19:00 h). Parentheses indicate the number of animals that survived.

Table 1
Comparison of parameters for periodic regression curves between*ad libitum* and 1-h feeding groups in male and female mice.

	Ad libitum feeding group					1-h feeding group					ICC		
	n	R^2	MESOR	Amp.	Phase	n	R^2	MESOR	Amp.	Phase	Amp.	Phase	Overall
Male mice													
Pre-level	9	0.694	21.583	18.242	00:46	10	0.765	17.342	16.826	22:43	0.997	0.858	0.855
Day 1	9	0.716	21.282	24.610	23:53	10	0.698	29.925	28.915	21:47	0.987	0.854	0.843
Day 2	9	0.834	30.051	30.850	00:18	10	0.366	53.063	23.705	22:02	0.966	0.828	0.800
Day 3	9	0.674	35.324	36.502	23:48	10	0.205	63.538	21.247	01:26	0.870	0.910	0.792
Female mice													
Pre-level	8	0.584	39.602	30.254	00:18	7	0.548	42.573	33.252	00:31	0.996	0.998	0.994
Day 1	8	0.530	44.120	38.072	00:03	7	0.165	93.655	14.058	09:09	0.650	−0.724	−0.470
Day 2	8	0.478	48.833	35.725	01:11	7	0.185	56.708	18.180	01:30	0.808	0.997	0.806
Day 3	8	0.580	52.411	38.472	00:45	6	0.083	13.993	5.859	00:11	0.298	0.989	0.294

[a] $p < 0.01$, significantly different from the corresponding *ad libitum* feeding group.

period. As shown in Table 1, ICC between the *ad libitum* and 1-h feeding groups revealed that acrophase was reversed by 1-h feeding. On day 2, there were no group differences in wheel-running extent or pattern. However, on day 3, both wheel-running extent ($F_{(1, 42)} = 42.687$, $p < 0.01$) and pattern ($F_{(2, 42)} = 7.726$, $p < 0.01$) differed among groups. On day 1, MESOR of 1-h feeding group was significantly higher than pre-level, while on day 3, the 1-h feeding group showed a significantly lower MESOR value compared with the pre-level. During the adaptation periods, there were no group differences in wheel-running extent (MESOR) or pattern, in either male or female mice.

Fig. 2 displays the periodic regression curves of female mice during the (a) adaptation and (b) experimental periods. As mentioned above, in the *ad libitum* feeding group the coefficient (R^2) was well-fitted with 24-h power density, but not in the 1-h feeding group. By contrast, in male mice, matching coefficients were consistent between the 1-h and *ad libitum* feeding groups (Table 1). Disruption of circadian rhythms following restricted feeding was more pronounced in female vs. male mice. Significant acrophase differences, between the *ad libitum* and 1-h feeding groups, were observed only on day 1. Based on these findings, evaluated drug effects in female mice using wheel-running rhythms after 1-h feeding.

Table 2 shows changes in body weight during the experimental period. Factorial ANOVA (4 × 4) revealed a significant difference between male and female mice ($F_{(3, 30)} = 46.258$, $p < 0.01$) and among trials ($F_{(3, 90)} = 199.264$, $p < 0.01$). Multiple comparisons showed that there were significant differences between males and females in both the *ad libitum* and 1-h feeding groups ($p < 0.01$). Compared with the pre-level, significant differences were found on day 1 ($p < 0.05$), day 2 ($p < 0.01$) and day 3 ($p < 0.01$) in male and female mice. Weight loss in male mice was 4.4% of pre-level on day 1 and 4.1% in female mice. On day 3, body weight loss was 24.1% of pre-level in male mice and 25.4% in female mice, with one death.

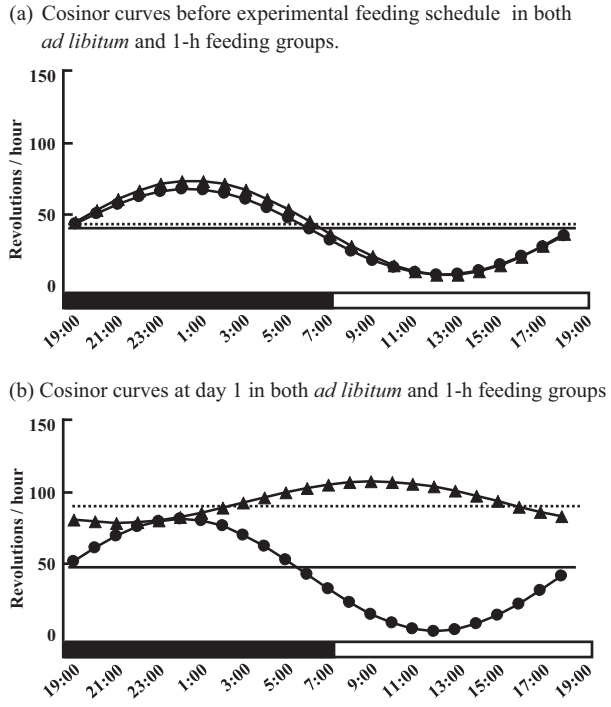

Fig. 2. Cosinor curves for the first day of the 1-h feeding schedule in female mice. The upper panel (a) shows periodic regression curves for the day before the experimental feeding schedule, and the lower panel (b) shows cosinor curves for the first day of the experimental feeding schedule. Circles (●) indicate the *ad libitum* feeding group and triangles (▲) indicate the 1-h feeding group. Solid line shows MESOR for the *ad libitum* feeding group and dotted line shows MESOR for the 1-h feeding group.

Table 2
Comparison of the body weight (g) between male and female mice or between *ad libitum* and 1-h feeding groups.

Groups	n	Pre-level	1-h feeding period		
			Day 1	Day 2	Day 3
Ad libitum feeding groups					
Male	9	38.0 ± 2.8	37.7 ± 2.9	37.8 ± 3.1	37.9 ± 2.8
Female	8	28.1 ± 1.4	28.3 ± 1.3	28.4 ± 1.2	28.8 ± 1.1
1-h feeding groups					
Male	10	38.2 ± 3.1	33.8 ± 2.9	30.7 ± 2.9	29.0 ± 3.2
Female	7	28.7 ± 2.3	24.6 ± 2.0	22.9 ± 2.0	21.6 ± 2.0

Each value indicates the mean ± S.E.
[a] $p < 0.01$, significantly different between male and female mice.
[b] $p < 0.05$ and [c]$p < 0.01$, significantly different from the corresponding pre-level.

3.2. Subchronic effects of antidepressant drugs

Fig. 3 illustrates the effects of imipramine and paroxetine on the disruption of circadian activity rhythms induced by the 1-h schedule in female mice. PANCOVA indicated no significant group differences in the extent ($F_{(5, 126)} = 0.709$) or pattern ($F_{(10, 126)} = 0.929$) of wheel-running during adaptation periods. On day 1 of the 1-h feeding schedule, significant differences among the six groups were observed in the extent ($F_{(5, 126)} = 22.604$, $p < 0.01$) and pattern ($F_{(10, 126)} = 10.616$, $p < 0.01$) of wheel-running (the *ad libitum* feeding group was added as a control). As shown in Table 3, compared with vehicle on day 1, Bonferroni's multiple comparisons revealed that imipramine and paroxetine treatment significantly decreased MESOR ($p < 0.01$). To evaluate the effect of drug on wheel-running pattern, we compared the activity consistency of the *ad libitum* and drug-treated groups using ICC. Compared with the vehicle group, the antidepressant groups were characterized by a higher matching coefficient, even under the 1-h feeding schedule.

3.3. Subchronic effects of tandospirone and DOI

Fig. 4 illustrates the effects of tandospirone and DOI on the disruption of circadian activity rhythms induced by the 1-h feeding schedule in female mice. Because of the different routes of administration, we used two vehicle-treated groups, and added an *ad libitum* feeding control group for analysis purposes. In the tandospirone-treated group, there was a significant difference in wheel-running extent ($F_{(3, 84)} = 5.741$, $p < 0.01$), but not pattern ($F_{(6, 84)} = 0.947$) during adaptation periods. As shown in Table 4, tandospirone at 2.5 mg/kg significantly decreased MESOR compared with the vehicle-treated group. On day 1 of the 1-h feeding schedule, there were significant differences among the four groups differences in the extent ($F_{(3, 84)} = 27.400$, $p < 0.01$) and pattern of wheel-running ($F_{(6, 84)} = 3.416$, $p < 0.01$). Compared with vehicle-treated mice, Bonferroni's multiple comparisons indicated that tandospirone treatment significantly decreased MESOR. To evaluate wheel-running pattern, we compared ICC values. Compared with the vehicle-treated group, tandospirone treatment was associated with a higher ICC value on day 1. However, DOI significantly affected only the extent of wheel-running. PANCOVA revealed significant group differences in extent on days 1 ($F_{(3, 84)} = 29.716$, $p < 0.01$), but not in pattern. As shown in Table 4, compared with vehicle, DOI was associated with significantly lower MESOR values ($p < 0.01$, respectively).

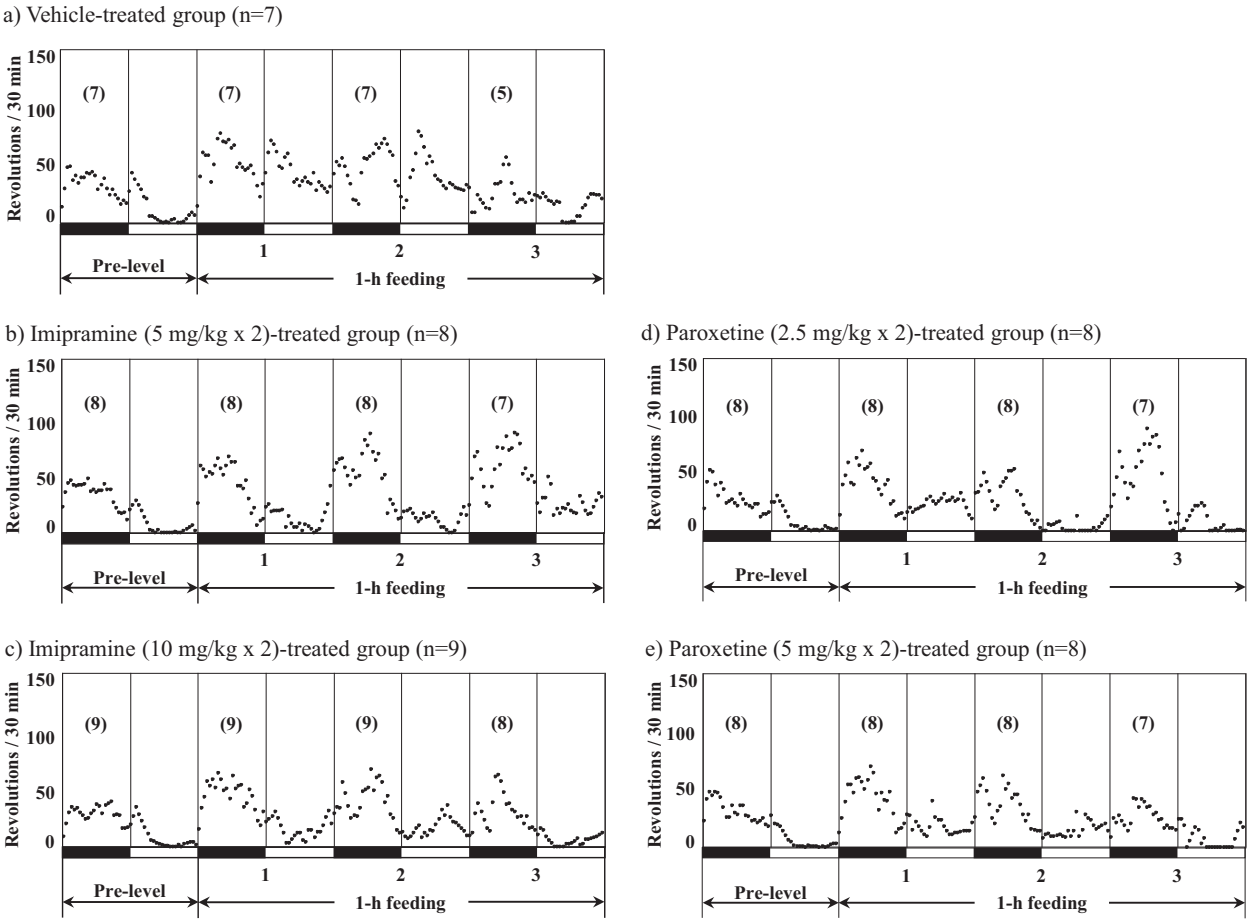

Fig. 3. Subchronic effects of imipramine and paroxetine on the disruption of the circadian running rhythm induced by 1-h feeding in female mice. Each dot shows the mean number of wheel revolutions per 30 min. Black rectangular columns show the dark phase (19:00–07:00 h) and white rectangular columns show the light phase (07:00–19:00 h). Parentheses indicate the number of animals that survived. The panels show (a) the 1-h feeding group treated with vehicle, (b) the 1-h feeding group treated with 5 mg/kg imipramine, (c) the 1-h feeding group treated with 10 mg/kg imipramine, (d) the 1-h feeding group treated with 2.5 mg/kg paroxetine, and (e) the 1-h feeding group treated with 5 mg/kg paroxetine. Drug or vehicle was administered twice per day (at 09:00 and 17:00 h).

3.4. Drug effects on body weight during 1-h feeding period

On day 1, ANCOVA showed that there was a significant effect among imipramine (5 and 10 mg/kg), paroxetine (2.5 and 5 mg/kg) and the vehicle groups ($F_{(4, 34)} = 3.511$, $p < 0.05$) (Table 5). Multiple comparisons indicated that there was a significant difference in body weight loss between the vehicle and 5 mg/kg imipramine groups or the 5 mg/kg paroxetine groups ($p < 0.05$, respectively). Body weight loss in the vehicle group was 16.6% compared with pre-levels, while the 5 mg/kg imipramine group was 11.5% and the

Table 3
Effects of imipramine and paroxetine on parameters for periodic regression curves in female mice.

Groups	n	R2	Parameters			ICC		
			MESOR	Amp.	Phase	Amp.	Phase	Overall
Vehicle (distilled water)								
Pre-level	7	0.664	42.325	35.837	01:05	0.986	0.978	0.964
Day 1	7	0.410	105.607	28.542	13:25	0.960	−0.937	−0.900
Imipramine 5 mg/kg								
Pre-level	8	0.877	40.206	41.177	00:28	0.954	0.999	0.953
Day 1	8	0.809	55.000	51.640	23:24	0.955	0.986	0.941
Imipramine 10 mg/kg								
Pre-level	9	0.802	35.749	33.091	01:46	0.996	0.927	0.923
Day 1	9	0.877	59.213	43.367	00:02	0.981	1.000	0.981
Paroxetine 2.5 mg/kg								
Pre-level	8	0.510	35.402	28.308	00:24	0.998	1.000	0.997
Day 1	8	0.497	62.328	28.940	22:16	0.964	0.893	0.860
Paroxetine 5 mg/kg								
Pre-level	8	0.711	37.125	36.311	00:17	0.984	1.000	0.984
Day 1	8	0.652	59.438	38.518	23:54	1.000	0.999	0.999

[a] $p < 0.01$, significantly different from the vehicle-treated group.

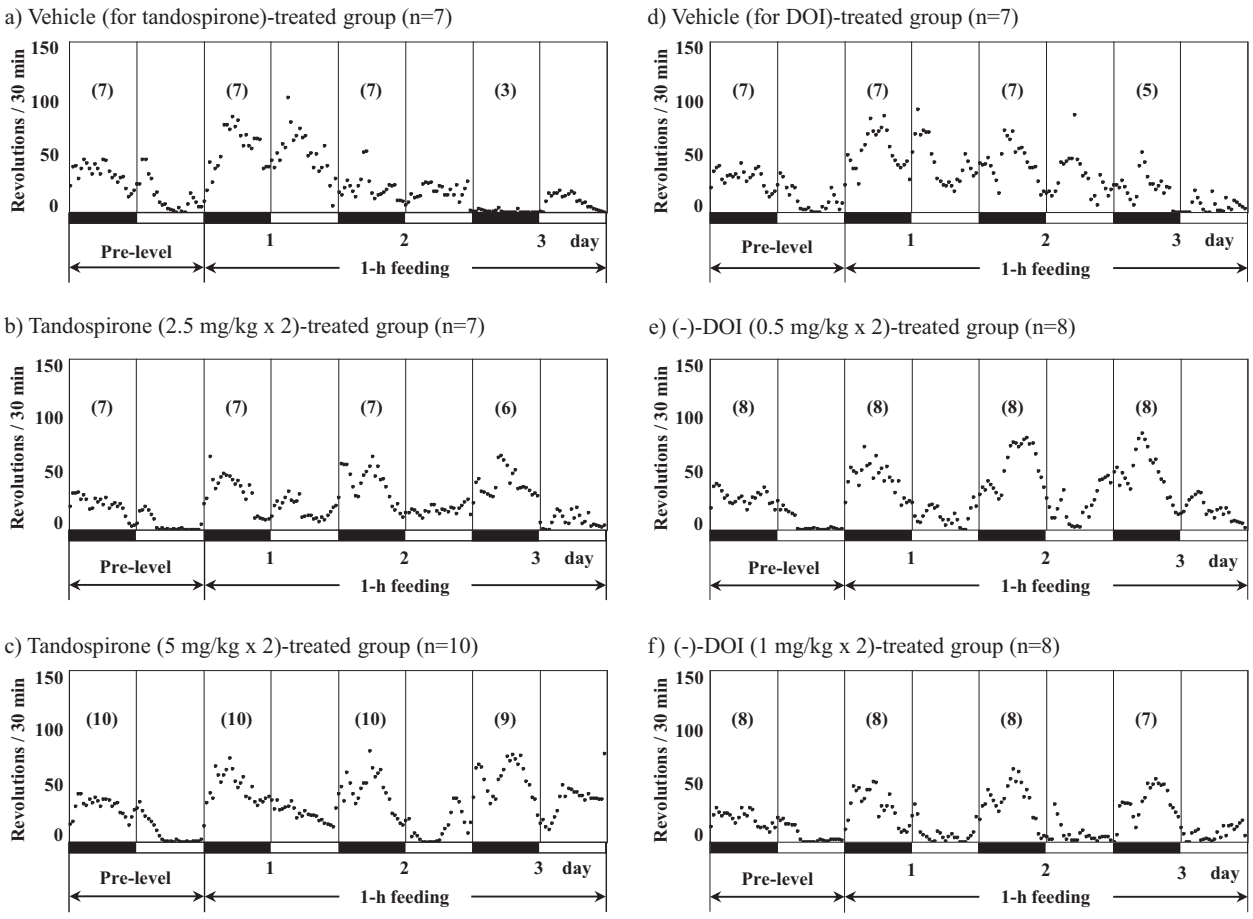

Fig. 4. Subchronic effects of tandospirone and (−)-DOI on the disruption of the circadian running rhythm induced by 1-h feeding in female mice. Each dot shows the mean number of wheel revolutions per 30 min. Black rectangular columns show the dark phase (19:00–07:00 h) and white rectangular columns show the light phase (07:00–19:00 h). Parentheses indicate the number of animals that survived. The panels show (a) treatment with the vehicle for tandospirone (twice/day), (b) treatment with 2.5 mg/kg tandospirone (twice/day), (c) treatment with 5 mg/kg tandospirone (twice/day), (d) treatment with the vehicle for (−)-DOI, (e) treatment with 0.5 mg/kg (−)-DOI (twice/day), and (f) treatment with 1 mg/kg (−)-DOI (twice/day).

5 mg/kg paroxetine was 12.4%. There were no significant differences between tandospirone (2.5 and 5 mg/kg) and the vehicle groups ($F_{(2, 20)} = 1.101$, N.S.) on day 1. ANCOVA revealed a significant effect among the (−)-DOI (0.5 and 1 mg/kg) and vehicle groups ($F_{(2, 19)} = 7.042$, $p < 0.01$) on day 1. There was also a significant difference in body weight loss between the vehicle (17.5%) and 1 mg/kg (−)-DOI groups (10.3%) ($p < 0.01$).

Table 4
Effects of tandospirone and (−)-DOI on parameters for periodic regression curves in female mice.

Groups	n	R^2	Parameters			ICC		
			MESOR	Amp.	Phase	Amp.	Phase	Overall
Vehicle (distilled water)								
Pre-level	7	0.595	46.791	32.483	01:09	0.998	0.975	0.973
Day 1	7	0.211	106.232	23.649	05:27	0.896	0.159	0.142
Tandospirone 2.5 mg/kg								
Pre-level	7	0.655	26.564	25.593	00:14	0.986	1.000	0.986
Day 1	7	0.430	50.458	25.497	23:03	0.925	0.965	0.892
Tandospirone 5 mg/kg								
Pre-level	10	0.793	38.833	36.574	01:29	0.982	0.952	0.935
Day 1	10	0.657	71.467	32.893	00:48	0.989	0.981	0.971
Vehicle (saline)								
Pre-level	7	0.720	41.108	30.388	00:55	1.000	0.986	0.986
Day 1	7	0.398	96.839	32.315	02:57	0.987	0.725	0.716
(−)-DOI 0.5 mg/kg								
Pre-level	8	0.722	33.736	31.261	01:17	0.999	0.967	0.966
Day 1	8	0.830	57.052	48.479	23:56	0.971	0.999	0.971
(−)-DOI 1 mg/kg								
Pre-level	8	0.648	26.693	22.153	01:04	0.953	0.979	0.933
Day 1	8	0.722	38.422	38.787	23:53	1.000	0.999	0.999

[a] $p < 0.01$, significantly different from the corresponding vehicle-treated group.

4. Discussion

The present study used chronometric analysis to demonstrate a 24-h circadian rhythm during wheel-running activity in both male

Table 5
Changes in body weight during 1 -h feeding period.

Groups	% Change		
	Day 1	Day 2	Day 3
Vehicle (distilled water)	−16.6 ± 1.3	−24.4 ± 0.5	−29.0 ± 0.9
Imipramine 5 mg/kg	−11.5 ± 0.7	−18.1 ± 1.5	−22.3 ± 1.8
Imipramine 10 mg/kg	−13.0 ± 1.1	−19.4 ± 1.6	−24.4 ± 2.2
Paroxetine 2.5 mg/kg	−14.7 ± 1.8	−18.9 ± 1.5	−21.4 ± 2.0
Paroxetine 5 mg/kg	−12.4 ± 1.4	−17.5 ± 1.8	−20.8 ± 1.5
Vehicle (distilled water)	−15.9 ± 1.5	−20.6 ± 1.8	−25.1 ± 1.7
Tandospirone 2.5 mg/kg	−12.6 ± 1.1	−19.2 ± 1.5	−22.5 ± 1.7
Tandospirone 5 mg/kg	−14.6 ± 1.1	−19.0 ± 1.1	−24.0 ± 1.4
Vehicle (saline)	−17.5 ± 2.2	−24.3 ± 1.2	−27.9 ± 0.5
(−)-DOI 0.5 mg/kg	−12.8 ± 0.3	−20.4 ± 1.1	−26.4 ± 1.7
(−)-DOI 1 mg/kg	−10.3 ± 1.1	−16.2 ± 1.6	−19.8 ± 1.8

Each values indicates % change (mean ± S.E.) comparing with the corresponding pre-level.
[a] $p < 0.05$. [b] $p < 0.01$, significantly different from the corresponding vehicle-treated group.

and female mice under *ad libitum* feeding conditions. Running activity rhythms were well-fitted to a theoretical cosigner curve, synchronizing with the 12-h light–dark cycle (lights on/off at 07:00/19:00). In both the male and female control groups, 75% of the wheel-running activity occurred during the dark phase, peaking at midnight, whereas mice were inactive during the light phase aside from immediately after light onset. This is consistent with previous reports of 24-h periodicity in the running-wheel rhythms of ICR male mice reared under a 12/12-h light–dark cycle (27). These authors also reported that a short bout of activity occurred within 1 h of light onset.

Following the 4-day adaptation period, restricted feeding disrupted the circadian rhythm synchronized with the light–dark cycle. In particular, starvation in female mice was rapidly reflected in running activity augmentation during dark and light phases, and consequent desynchronization of the circadian wheel-running rhythm with the light–dark cycle. By contrast, the wheel-running revolutions of male mice gradually increased on 1-h feeding schedule days 2 and 3. This gender difference indicates that female mice may be more susceptible to food deprivation. In an early study by Páre et al. (28), wheel running-activity was significantly greater in female vs. male rats; survival time under 1-h feeding conditions was also longer in male rats. We obtained similar results: the daily number of wheel revolutions in ICR mice was higher in females (950.4 ± 74.2, $n = 7$) vs. males (518.0 ± 115.0, $n = 10$). Although the body weight of female mice was significantly less than that of males, the rate of weight loss following 1-h feeding exhibited similar patterns. However, males survived for longer: on day 5, one female had survived, compared with six males. It is conceivable that differences in baseline wheel-running activity may involve differences in food deprivation susceptibility. The running activity of female mice could also have varied with the estrous cycle during the experimental period, because the estrous cycle in female mice is approximately 4.5 days. We did not measure the estrous cycle after mice were placed into the activity wheel cage, because frequent vaginal smear collections induce pseudopregnancy in small rodents. However, to eliminate potential spontaneous activity fluctuations during the estrous cycle (29, 30), we used only female mice in the diestrus stage immediately before introducing them to the running-wheel cage.

An important finding was that subchronic treatment with imipramine or paroxetine prevented circadian wheel-running rhythm desynchronization under 1-h feeding conditions. As illustrated in Figs. 3 and 5, the circadian rhythms of female mice treated daily with imipramine (at 5 or 10 mg/kg, twice per day) or paroxetine (at 2.5 or 5 mg/kg, twice per day) were more-clearly delineated. In a preliminary experiment, we attempted the acute administration of these antidepressant drugs after desynchronization, but neither imipramine nor paroxetine affected circadian rhythm desynchronization. In addition to its role in clinical practice (31, 32), repetitive drug treatment may also mediate the efficacy of antidepressant drugs. Consistent with our results, the effects of tricyclic antidepressants (clomipramine, desipramine and imipramine) on circadian corticosterone rhythms depend on the drug administration regimen (acute or chronic) employed (33). Because the onset of wheel-running rhythm desynchronization in female mice occurred on day 1 of the 1-h feeding schedule, we commenced drug (or vehicle) administration on the day that mice were placed into the running-wheel cage. During the adaptation period, compared with the running activity rhythms of the *ad libitum* feeding group, neither imipramine nor paroxetine was associated with differences in the extent or pattern of wheel-running. In addition, as we previously reported (34, 35), neither imipramine nor paroxetine impaired the spontaneous motor activity of female mice. Weber et al. (36) also reported that 30 mg/kg imipramine significantly suppressed wheel-running but 10 mg/kg imipramine had no effect. Therefore, the possibility that these drug effects might be concealed by drug-induced alterations in motor behavior can be discounted. However, it remains unclear why antidepressant drugs prevent the disruption of circadian running activity rhythms induced by a 1-h feeding schedule. Because subchronic treatment with imipramine or paroxetine showed lower

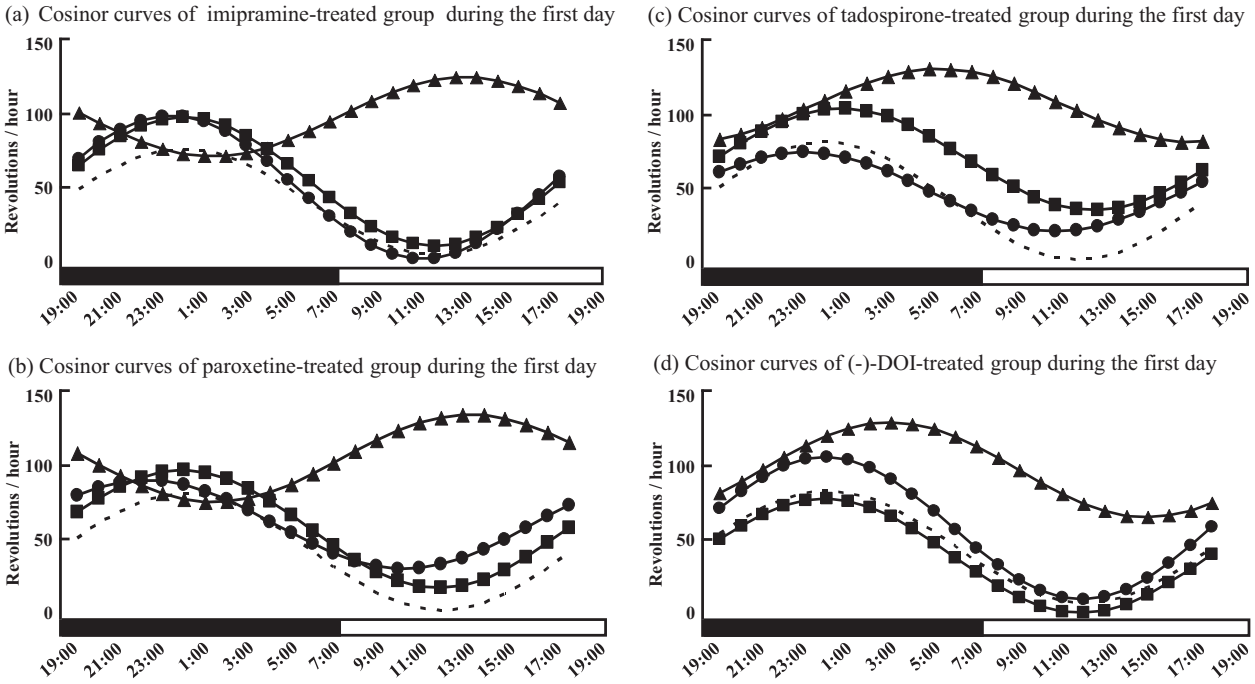

Fig. 5. Cosinor curves for the first day of the 1-h feeding schedule in female mice following drug or vehicle treatment. The panels show (a) the imipramine-treated group, (b) the paroxetine-treated group, (c) the tandospirone-treated group, and (d) the (−)-DOI-treated group. The dotted line indicates the *ad libitum* feeding group. ▲: vehicle; ●: low-dosage group; ■: high-dosage group.

loss of body weight than that of the vehicle group, we cannot eliminate the possibility that these drugs might be involved in drug-induced prevention of weight loss.

Concerning the mechanisms underlying the disruption of circadian running rhythms induced by restricted feeding, we also investigated whether stimulating serotonergic receptors alters these rhythms. Starting with an early series of studies by Nagayama and his colleagues (37–39), it has been well-documented that brain 5-hydroxytryptamine (5-HT) receptor activity closely correlates with the modulation of a number of circadian rhythms. Hellhammer et al (40) reported that rats housed individually in running-wheel cages, under 1-h feeding schedules, exhibit decreased 5-HT levels in the midbrain and cortex, in addition to increased levels in the medulla oblongata. Therefore, we also investigated whether stimulating 5-HT-receptors can prevent the circadian rhythm disruption induced by a 1-h feeding schedule, using two different agonists. Our results clearly demonstrate that subchronic treatment with tandospirone (a 5-HT_{1A}-receptor agonist) and (−)-DOI (a 5-HT_{2A}-receptor agonist) prevented the running activity rhythm disruptions induced by a 1-h feeding schedule (Figs. 4 and 5). It is likely that a 1-h feeding schedule will cause decreased 5-HT receptor signaling; either 5-HT_{1A} or 5-HT_{2A} agonists could compensate for this dysfunction by stimulating 5-HT receptors. However, the dose-dependent effects of tandospirone were not clearly discernible in our study, whereas (−)-DOI prevented circadian rhythm disruption in a dose-dependent manner. Chronic treatment with tandospirone (41) or (−)-DOI (24), at the doses employed herein, did not affect spontaneous motor activity. Consequently, the dysfunction in serotonergic nervous activity induced by restricted feeding might disrupt the circadian wheel-running rhythms of female mice. Interestingly, both the suprachiasmatic nucleus and pineal body, which act as circadian rhythm pacemakers, are densely innervated by serotonergic neurons (42), and 5-HT_{1A} and 5-HT_7 receptors play an essential role in setting the mammalian circadian clock (8, 43, 44). However, because (−)-DOI and antidepressants significantly prevented the loss of body weight induced by 1-h feeding in the present study, a different mechanism may underlie the preventive effect of (−)-DOI on behavioral rhythm in female mice.

Rats and mice reared individually in running-wheel cages, and fed only during a 1-h period each day, exhibited a marked increase in running activity and weight loss, followed by death. This well-known phenomenon was first termed “self-starvation” (10, 11) and subsequently renamed “activity-stress” by Páre and Houser (12); these authors emphasized that hyperactivity itself generated stress. Ethologically, in a variety of animal species (from insects to primates), food scarcity is known to cause dispersal (one-way movement) from less-to-more favorable habitats (45). Taking field observations into consideration, a mouse may maintain a high level of running activity throughout the day and night until it obtains sufficient food; if this goal-directed behavior does not yield satisfactory consequences, the mouse is likely to have exhausted the energy required to live. Because circadian rhythm alterations accompany depressive mood states (3), and because the experimental situation (involving food and social deprivation) is inescapable and therefore similar to learned helplessness (46) or despair (47), we suggest that circadian activity rhythm alterations in female mice, induced by a 1-h feeding schedule, may be a useful for investigating the psychopathology of depression.

Conflicts of interest

There is no conflict of interest in this study.

References

1 Parry BL, Newton RP. Chronobiological basis of female-specific mood disorders. Neuropsychopharmacol. 2001;25:S102–S108.
2 Maurizio F. Daytime sleepiness and insomnia as correlates of depression. J Clin Psychi. 2004;65:27–32.
3 Salvatore P, Indic P, Murray G, Baldessarini RJ. Biological rhythms and mood disorder. Dialogues Clin Neurosci. 2012;14:369–379.
4 Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, et al. Gender differences in chronic major and double depression. J Affect Dis. 2000;60:1–11.
5 Casper RC. Depression and eating disorders. Depress Anxiety. 1998;8(Suppl. 1):96–104.
6 Nagayama H. Influences of biological rhythms on the effects of psychotropic drugs. Psychosom Med. 1999;61:618–629.
7 Ohdo S. Circadian rhythms in the CNS and peripheral clock disorderes: chronopharmacological findings on antitumor drugs. J Pharmacol Sci. 2007;103:155–158.
8 Tahara Y, Shibata S. Chrono-biology, chrono-pharmacology, and chrono-nutrition. J Pharmacol Sci. 2014;124:320–335.
9 Hall JF, Hanford PV. Activity as a function of a restricted feeding schedule. J Comp Physiol Psychol. 1954;47:362–363.
10 Routtenberg A, Kuznesof AW. Self-starvation of rats living in activity wheels on a restricted feeding schedule. J Comp Physiol Psychol. 1967;64:414–421.
11 Routtenberg A. “Self-starvation” of rats living in activity wheels: adaptation effects. J Comp Physiol Psychol. 1968;66:234–238.
12 Páre WP, Houser VP. Activity and food-restriction effects on gastric glandular lesions in the rat: the activity-stress ulcer. Bull Psychon Soc. 1973;2:213–214.
13 Páre WP. Feeding environment and the activity-stress ulcer. Bull Psychon Soc. 1974;4:546–548.
14 Páre WP. Activity-stress ulcer in the rat: frequency and chronicity. Physiol Behav. 1975;16:699–704.
15 Tsuda A, Tanaka M, Nishikawa T, Iimori K, Hoaki Y, Ida Y, et al. Influence of feeding situation on stomach ulcers and organ weights in rats in the activity-stress ulcer paradigm. Physiol Behav. 1981;28:349–352.
16 Páre WP, Schimmel GT. Stress ulcer in normotensive and spontaneously hypertensive rats. Physiol Behav. 1986;36:699–705.
17 Watanabe K, Hara C, Ogawa N. Relationship between running activity rhythm and the development of activity-stress ulcer in rats. Jpn J Pharmacol. 1990;52:421–429.
18 Monteleone P, Martiadis V, Maj M. Circadian rhythms and treatment implications in depression. Prog Neuropsyhopharmacol Biol Psychiatry. 2001;35(7):1569–1574.
19 Courtet and Olie E. Circadian dimension and severity of depression. Eur Neuropsychopharmacol. 2012;22(Suppl. 3):S476–S481.
20 Goodnick PJ, Chaudry T, Artadi J, Arcey S. Women's issues in mood disorders. Expert Opin Pharmacother. 2000;1:903–916.
21 Sloan K, Kornstein SG. Gender differences in depression and response to antidepressant treatment. Psychiatr Clin North Am. 2003;26:581–594.
22 Duncan Jr WC. Circadian rhythms and the pharmacology of affective illness. Pharmacol Ther. 1996;71(3):253–312.
23 Lucki I, Singh A, Kreiss DS. Antidepressnt-like behavioral effects of serotonin receptor agonists. Neurosci Biobehav Rev. 1994;18:85–95.
24 Bekku N, Yoshimura H, Araki H. Factors producing a menopausal depressive-like state in mice following ovariectomy. Psychopharmacol Berl. 2006;187:170–180.
25 Nelson W. Methods for cosinor-rhythmometry. Chronobiologia. 1979;6(4):305–323.
26 Hayashi H, Hatano K, Tsuda M, Kanematsu K, Yoshikane M, Saito H. Relationship between circadian variation of ambulatory blood pressure and age or sex in healthy adults. Hypertens Res. 1992;15:127–135.
27 Ticher A, Ashkenazi IE. Compound structure of rodents activity rhythm. Physiol Behav. 1995;57:37–40.
28 Páre WP. Sex differences and incidence of activity-stress ulcers in the rat. Psychol Reports. 1978;43:591–594.
29 Finger FW. Estrus and general activity in the rat. J Comp Physiol Psychol. 1969;68:461–470.
30 Guttman R, Lieblich I, Gross R. Behavioral correlates of estrous cycle stages in laboratory mice. Behav Biol. 1975;13:127–132.
31 Anderson IM, Tomenson BM. The efficacy of selective serotonin reuptake inhibitors in depression: a meta analysis of studies against tricyclic antidepressants. J Psychopharmacol. 1994;8:238–249.
32 Gelenberg AJ, Chesen CL. How fast are antidepressants? J Clin Psychiatr. 2000;61:712–721.
33 Gómez F, Grauġes P, Martin M, Armario A. The effect of chronic administration of antidepressants on the circadian pattern of corticosterone in the rat. Psychophamacol Berl. 1998;140:127–134.
34 Bekku N, Yoshimura H. Animal model of menopausal depressive-like state in female mice: prolongation of immobility time in the forced swimming test following ovariectomy. Psychopharmacol Berl. 2005;183:300–307.
35 Su J, Hato-Yamada N, Araki H, Yoshimura H. A test-retest paradigm of the forced swimming test in female mice is not valid for predicting antidepressant-like activity: participation of acetylcholine and sigma-1 receptors. J Pharmacol Sci. 2013;123:246–255.

36 Weber M, Talmon S, Schlze I, Boeddinghaus C, Gross G, Schoemaker H, et al. Running wheel activity is sensitive to acute treatment with selective inhibitors for either serotonin or norepinephrine reuptake. Psychopharmacol Berl. 2009;203:753–762.
37 Nagayama H, Lu J-Q. Circadian rhythm in the responsiveness of central 5-HT_{2A} receptor to DOI in rats. Psychopharmacol Berl. 1996;127:113–116.
38 Lu J-Q, Nagayama H. Circadian rhythm in the response of central 5-HT_{1A} receptors to 8-OH-DPAT in rats. Psychopharmacol Berl. 1996;123:42–45.
39 Nagayama H, Lu J-Q. Circadian and circannual rhythms in the function of central 5-HT_{1A} receptors in laboratory rats. Psychopharmacol Berl. 1998;135:279–283.
40 Hellhammer DH, Hingtgen JN, Wade SE, Shea PA, Aprison MH. Seronergic changes in specific areas of rat brain asociated with activity-stress gastric lesions. Psychosom Med. 1983;45:115–122.
41 Yoshimura H, Ogawa N. Ethopharmacology of maternal aggression in mice: effects of diazepam and SM-3997. Eur J Pharmacol. 1991;200:147–153.
42 Morin LP. Serotonin and the regulation of mammalian circadian rhythmicity. Ann Med. 1999;31:12–33.
43 Yokota S, Moriya T, Shibata S. Inhibitory action of 5-HT1A agonist MKC-242 on triazolam-induced phase advances in hamster circadian activity rhythms. J Pharmacol Sci. 2005;98:103–106.
44 Horikawa K, Fuji K, Fukazawa Y, Shibata S. Two distinct serotonin receptors comediate non-photic signals to the circadian clock. J Pharmacol Sci. 2013;123:402–406.
45 Dingle H. Migration: the biology of life on the move. England. 2nd ed. Gordon's Oxford University Press; 2014.
46 Seligman ME. Learned helplessness. Annu Rev Med. 1972;23:407–412.
47 Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn. 1977;229:327–336.